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ISOLATION, PURIFICATION AND MODIFICATION OF PAPAIN ENZYME TO ASCERTAIN INDUSTRIALLY VALUABLE NATURE

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ABSTRACT

Papaya (*Carica papaya* L.) is one of the most popular and economically significant fruit highly prevalent in both the tropical and subtropical arena of the world such as Hawaii, South Pacific, South America, East Africa and South East Asia. Not only this item is consumed as fresh fruit or vegetable but also utilized as potential enzyme and nutrients supplement in food industry. Unripe green papaya as well as other part of papaya tree is concentrated with latex, which is composed of several valuable enzymes: papain, chymopapain, caricain and glycyl endopeptidase. To make industrially useable state, these enzymes needs to extracted and purified from green papaya fruits. It is crucial to isolate papain in the native crystalline state from fresh latex. Different well established methods of isolation and purification of crystalline papain are described here. ATPS and Sephadex G-75 based methods followed by several drying procedure are widely used for papain isolation and purification. To test and optimize the enzymatic activity, tyrosine method and Z-Gly-pNP are also frequently applied. Aided with several advance techniques, kinetics and ionization of catalytic site are also obtained. Finally, at the aim of high purity and enhanced catalytic activity a number of enzyme modifications approach has also been illustrated here. Recently, this enzyme is being used for pharmaceutical and medical purposes.

KEYWORDS: Latex, Papain, Proteolytic Enzymes, ATPS, Synthetic Substrate, Tyrosine, Z-Gly-pNP, Sephadex G-75

INTRODUCTION

Twenty first century is the era of biotechnology has spread its wings towards commercially valuable complicated biochemical processes. One of its major branches is enzyme technology that makes different industrial procedures convenient, economical and simple. 'Papain' is one of the product of this technology has diverse applications in chemical and food industries. Unripe papayas are the principal source of papain enzyme [1]. It carries proteolytic activity and belongs to cysteine proteinase family. Active papain enzymes can be isolated and purified from the latex of green papaya fruits [2, 3, 4].

The proteolytic activity of papain has been well described in the literature, including the degradation of elastin and proteoglycans [5]. According to Dr John and Whitman Ray Papain possesses a very powerful digestive action superior to pepsin and pancreatin. Recently Kinoshita in 2003 reported that Papain a major chemical compound extracted from latex of papaya used in several industry for various industrial and pharmaceutical products. This review focuses primarily on two aspects. Firstly, on understanding morphological properties of papain enzyme, composition and its isolation and modification method of papain isolation from papaya, and secondly on how these modification have advanced enzyme quality and activity. This is the comprehensive review on papain that attempts to integrate so many aspects of this economically important enzyme that should prove valuable for professionals involved in both research and commerce.

This study has been done to gather knowledge about papain enzyme and its isolation methods to build up this enzyme manufacturing industry in prospects for the new entrants.

PAPAIN ENZYME

The action of papain was first investigated by G.C. Roy in 1873 in an article published in the Calcutta Medical Journal entitled "The Solvent Action of Papaya Juice on Nitrogenous Articles of Food". Papain was first named in the late nineteenth century by Wurtz and Bouchut who partially purified the product from the sap of papaya[6]. When named, it was simply recognized as a proteolytically active constituent in the latex of tropical papaya fruit[7]. In the 1980s, the geometry of the active site was reviewed and the three-dimensional structure was determined to a 1.65 Angstrom resolution[8]. The precursors and inhibitors of papain were studied into the 1990s [9].

Properties of Papain

Principal properties of Papain enzymes are mentioned here according to the published work of Abhijit and Gadeker in 2007[10].

Alternate names = Papaya peptidase I.

Specificity = Cleaves somewhat nonspecifically at exposed residues.

Source = Carica papaya latex.

Storage conditions =Store at 4°c

Molecular weight =23.000 Da

Inhibitors = Heavy metals. Carbonyls, NEM, p-Chloromercuro-benzoate.

Extinction coefficient = 76,630 cm-1 M-1

Isoelectric point: pH 9.6

Catalytic residues of the enzymes are [11,12]:

- Cysteine (C158)
- Histidine (H292)
- Asparagine (N308).

Composition of Papain

Papain is a single-chained polypeptide with three disulfide bridges and a sulfhydryl group that are highly essential for the activity of the enzyme. Papain is expressed as an inactive precursor, prepropapain. For the formulation of an active papain requires several cleavage steps that include an initial cleavage of the 18 amino acid preregion followed by further cleavage of the glycosylated 114 amino acid proregion (9).This proregion serves as an intrinsic inhibitor and folding template[13, 14].

The Complete Amino Acid Sequence of Papain

The papain precursor protein contains 345 amino acid residues, and consists of a signal sequence (1-18), a propeptide (19-133) and the mature peptide (134-345). The amino acid numbers on papain are based on the mature

peptide. The protein is stabilized by the three disulfide bridges. Its three-dimensional structure consists of two distinct structural domains with a cleft between them[15, 16, 17)

Table 1: The Complete Amino Acid Composition of Papain

Amino Acid	No	Amino Acid	No
Lysine	10	Glycine	28
Histidine	2	Alanine	14
Arginine	12	Valine	18
Aspartic acid	7	Isoleusine	12
Asparagine	12	Leusine	11
Glutamic	8	Tyrosine	19
acid	12	Phenylalanine	4
Glutamine	8	Tryptophan	5
Threonine	13	Cysteine	1
Serine Proline	10	Half cysteine	6

The active site, consisting of a cysteine and a histidine, lies at the surface of the cleft. Apart from four short α-helical segments and one short segment of β-structure, the conformation of the chain is irregular[18].

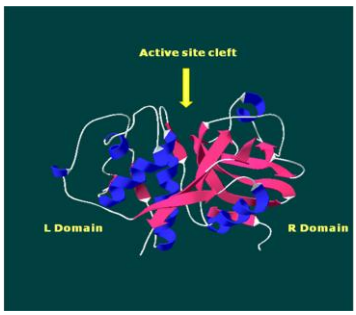

Figure 1: Ribbon Structure of Papain Enzyme

ISOLATION OF PAPAIN ENZYME

Collection and Extraction of Latex

A fully green unripe and mature papaya fruits is collected in the early morning and then by cutting the skin extracted white milky latex [4,19]. Tapping of the fruit started early in the morning and finish by mid-late morning (i.e. during periods of high humidity). At low humidity the flow of latex is low. The incisions are made using a stainless steel razor blade or wooden sharp knives .The blade should not protrude more than about 2 mm as cuts deeper than 2 mm risk juices and starch from the fruit pulp mixing with the latex that lowers the quality. Fruits should be tapped at intervals of about 4-7 days and for the first tapping it is usually sufficient to make only one cut. Latex is collected in stainless steel trays while latex coagulated in the surface of the fruits is scrapped and collected in the trays. The use of a close fitting lid and keeping the box in the shade are both important because they reduce the reactions which cause the loss of enzyme activity[20]. The extracted latex also obtained by several longitudinal incisions with a rustless-steel blade on the unripe fruits using Nitsawang's protocol[21]. This latex can be allowed to run down the fruit and drip in plastic containers. Before being stored at -20ºC NaOH 0.3 M was added to avoid oxidation.The other used latex can be obtained from the unripe fruits skin, which are peeled and crushed in a food processor obtaining a humid paste. NaOH 0.3 M has to be added to this paste before being stored at - 20ºC [22].

Cleaning and Sieving

After collection latex is passed through mesh sieves to remove dirt and then the papaya latex is mixed with Potassium meta-bisulphate (Kms). Sieved to remove extraneous materials and then blended with activated zeocarbcation exchange resin. The mixture is then centrifuge to separate out the resin from liquefied latex and kept in cold storage to avoid fermentation [10].

PURIFICATION OF PAPAIN

According to Kimmel and Smith, Papain from papaya latex is routinely purified and this method involve extraction of the latex, removal of material insoluble in the extract at pH 9.0, an ammonium sulfate precipitation followed by three recrystallizations. The resulting protein contains three components, active papain, activate-able papain, and non-active-able papain[23]. In active papain, the thiol group is fully reduced. Active-able papain, which itself is inactive, can be converted to active papain by reaction with thiols. Klein and Kirsch presented convincing evidence which shows that, in active-able papain, the thiol group is in a disulfide linkage with the amino acid cysteine. Non active-able papain cannot be activated to an enzymatically active material by the addition of thiols[24]. The crude papain is purified by dissolving in water and precipitating with alcohol [25].

A Two-Step Procedure for Purification of Papain from Extract of Papaya Latex

For the purification of papain from extract of papaya latex' this procedure involves precipitation of the extract of papaya latex with sodium chloride followed by affinity chromatography of the re-dissolved precipitate. One procedure employs affinity chromatography on a column consisting of the inhibitor Gly-Gly-Tyr (Bzl)-Arg covalently linked to Sepharose[26]. The other procedure makes use of the presence of a reactive thiol group in active papain and the absence of such a thiol group in non-active-able papain [27]. In this method, active papain is separated from inactive papain on a column of Sepharose containing covalently linked p-aminophenol- mercury groups. Both methods yield active papain containing one thiol group per molecule of protein [28,29].

Purification of Papain with Aqueous Two-Phase System (ATPS)

Papain is purified by Aqueous Two-Phase System[30]. Their study showed that the separated papain was still contaminated with chymopapain. In 2006, Nitsawang reported the use of polyethylene glycol (PEG)-(NH4)2SO4 system for purifying papain from fresh papaya latex collected from the papaya fruit directly[21]. Mingliang in 2010 first prepare aqueous two-phase system for purification of Papain. Aqueous two-phase systems were prepared in a graduated tube with 4 g enzyme solution plus various amounts of PEG (4000 or 6000), salt solution (40% w/w phosphate or 40% w/w (NH4)2SO4) and deionized water to reach a total weight of 10 g. Phosphate solution was prepared using K2HPO4 and NaH2PO4, as they display greater solubility than their respective monobasic and dibasic salts. Monobasic and dibasic salt solutions were mixed to achieve a certain pH value. To ensure complete phase separation, the systems were centrifuged at 10,0006g for 15 min at respective temperature. Phase volumes were measured, and then aliquots of the phases were taken to determinate protein concentration and activity[31, 32]. The presence of papain was verified by Basic Protein Native-PAGE and FPLC. Then purity analysis is performed. Purity of the purified papain was evaluated by ion-exchange chromatography on FPLC (Fast protein liquid chromatography). All the top PEG phase samples were diluted to 1 mg/ml for the FPLC and the chromatographic separation was carried out using a gradient. The UV-900 detector was set at 280 nm for measuring the protein's aromatic residues. The elution peak of papain was confirmed by standard papain [33, 34]. Aqueous Two-Phase partition helps the procedure of the recovery of papain enzyme[35].

Sephadex G-75 Method

According to Rubens Monti and Carmelita, the latex was immediately used for the purification of papain in its native state or stored at -8°C protected under a nitrogen atmosphere after extraction. For extract preparation, Ethylene di-amine-tetra-acetic acid (EDTA), pH 7.0, was added to the fresh latex to a final concentration of 1 mM and the preparation was kept under nitrogen for 1 hour at room temperature with constant shaking. The suspension was then centrifuged at 12,000 x g for 30 min at room temperature in a Sorvall RC-2B centrifuge with an SS-34 rotor. Protein was measured by the method of Itzhaki and Gill (1964) and by absorbance at 280 nm. To determine the molecular weight of papain obtained by the method of using Sephadex G-75, the pure papain exhibited parent molecular masses of 21 kDa and the classical papain 21,3kDa, when G-75 Sephadex was used[36].

Purification of Papain from Fresh Latex of Carica Papaya

Balls developed a process for the purification and isolation of papain in the native crystalline state from fresh latex[11]. This method was later modified using commercially available dry latex and has been the classical method for papain preparation for many years, with some later modifications[23,37,38]. According to Brocklehurst aqueous extracts of Carica papaya latex contain some cysteine proteinases that can be separated by ion exchange chromatography, and fully active forms can also be obtained by covalent chromatography using thiol-disulfide exchange [1].

Drying Method

The method of drying is the main factor that determines the final quality of papain. Subsequently the latex is spread in trays and drying takes about 4 hours at 55°. Then the latex is scratched from the trays. It is again mixed with Kms and ground in SS hammers mills or roller and mills to get fine powder. According to Science tech entrepreneur in 2010 the drying is continued until the product comes off in flakes having a porous structure.

Sun drying gives the lowest quality product as there is considerable loss of enzyme activity and the papain can easily turn brown. However, in many countries sun drying is still the most common processing technique for papain. The latex is simply spread on trays and left in the sun to dry. The activity and quality of the sun dried crude papain is enhanced by using preservatives. Thepreservatives improve appearance/color smell and enzyme activity with respect to control sample. Treatment with 0.1% (W/V) Sodium meta-bisulfite gave the best result, benzoic acid at 0.1% improve appearance and color of the sun dried crude papain better than Sodium Benzoate but Sodium Benzoate gave the better activity enhancement [39].

Oven drying can be of simple construction. In Sri Lanka they are generally simple out-door stoves (about one meter high) made out of mud or clay bricks. Drying times vary but an approximate guide is 4-5 hours at a temperature of about 35-40°C. Drying is complete when the latex is crumbly and not sticky. A better quality product is obtained if the latex is sieved before drying. The dried product should be stored in air-tight and light-proof containers and kept in a cool place. Metal containers should be lined with polythene[39].

Spray drying is not possible at small-scale. Spray dried papain has a higher enzyme activity than other papain's and is totally soluble in water. Extreme care must be taken when handling this form of papain because it can cause allergies and emphysema if inhaled. For this reason spray dried papain is often encapsulated in a gelatin coat. Then its proteolytic activity is tested in laboratory[40].

A conventional oven (Memmert), a vacuum oven (Cole-Parmer 5053-20) and a lyophilizator (Freezone 6 plus Labconco) are used to dry the obtained latex and to establish the temperature effect on the crude enzyme

activity. Following table presents the operational conditions for the different dryers. To each condition three different assays were made.

Table 2: Operational Conditions for the Different Dryers Used in the Latex Drying

Number	Origin	Drier Type	Temp (○C)	Pressure (mbar)	Duration (h)
1	Latex	Oven	40	746.6	8
2	Skin	Oven	50	746.6	8
3	Latex	Tray Drier	40	746.6	2
4	Skin	Tray Drier	50	746.6	2
5	Latex	Vacuum Oven	40	137.06	18
6	Skin	Vacuum Oven	50	137.06	18
7	Latex	Lyophilizator	-30	0.1	24
8	Skin	Lyophilizator	-40	0.1	24

Principal Characteristics of Purified Papain

Purified papain will be white or grayish white, slightly hygroscopic powder. It is completely soluble in water and glycerol, and practically insoluble in most organic solvents. Its potency varies according to process of preparation. Papain can digest about 35 times its own weight of lean mean. The best quality papain digests 300 times its own weight of egg albumin. It should be kept in well-closed containers. The best pH for its activity is 5.0 but it functions also in neutral and alkaline media.The following standard is recommended for high quality papain:

- Color should be creamy white.
- Moisture content should be above 10 percent.
- Total ash content should not be greater than 11.1% on moisture free basis.
- Should not content any foreign substances.
- Should possess a proteolytic activity not less than that of ceylon reference [42].

DETERMINATION OF ENZYMATIC ACTIVITY

If papain is to be exploited commercially for an export market or local food industry use, it is important to be able to determine the enzymatic activity. The method used to measure the papain activity must be validated in order to certify the results. Papain enzymatic activity can be determined by the measurement of the hydrolysis of natural proteins or synthetic substrates, like esters or low molecular weight amides. Such reactions may release dyes or chormogenic or fluorogenic products, which can be measured by spectrophotometers or fluorimeter. Protease enzymatic activity was determined by Sigma's SSCASE01.001 protocol (1999). This uses casein as protease substrate. Dried samples of 0.05 g were dissolved in 5 mL sodium acetate buffer 10 mM (pH 7.5) and 5 mL calcium acetate buffer 10 mM (pH 7.5). For each sample 455 µL Casein 65% (w/v) were preheated in a thermal bath at 37±1ºC for 10 minutes and then 20 µL of these were added. After 10 min of reaction, the reactions were stopped by the addition of 455 µL tri-chloroacetic acid 110 mM and were kept in the thermal bath for another 30 min. The two form phases were separated by centrifugation at 9000 rpm and 4ºC during 20 min (Fresco 17 Thermo) in order to discard the solid formed. The supernatant was taken for protease assays. Aliquots of 625 µL supernatant were added to 1570 µL sodium carbonate 500 mM and 250 µL of Folin Ciocalteus reagent. The protease activity was detected spectrophotometrically since the released tyrosine developed a blue coloration. Each sample was read in a spectrophotometer at 660nm. The protein presented in each sample was determined by Biuret´s method [41].

N-CBZPHE-ARG-7-MCA Method

Papain (sample and/or standard) or formulations (containing or not papain) (20.0 μL) is mixed with N-CBZ-PHEARG-7-MCA substrate (125.0 μL); acetic acid 30% (v/v) and EDTA cysteine buffer (specifically to promote the reaction interruption (40.0 μL). During preparation of the micro-plate scheme, all solutions were kept in ice bath, including the plate and after the micro-plate was put in water bath at 40.0 ± 0.5 °C for 45 minutes and the reaction was interrupted in intervals of 15 minutes with acetic acid 30% (v/v) solution addition, initiating at time zero and finalizing at 45 minutes. Fluorescence was registered with filters giving excitation and emission wavelength at 360 and 460 nm and a curve of 4 time values was constructed for each sample aiming the determination of the increase velocity of fluorescence in function of papain concentration [43].

Tyrosine Method

Another method to determine the activity of a papain sample, a known amount of papain sample is mixed with a fixed amount of casein (the protein found in milk). The reaction is allowed to proceed for 60 minutes at 40°C. After this time the reaction is stopped by-the addition of a strong acid. The product of the reaction is known as tyrosine which is known to absorb ultra-violet light (invisible to the human eye). The solutions containing the tyrosine are prepared for analysis using the spectrophotometer. The amount of ultra-violet light absorbed by the solution can be related to the number of tyrosine units produced by the papain sample. Hence, the greater this number, the greater the activity of the papain[10]. Brill and Brown in 1922 state that the papain appears to loss its activity on keeping, a sample examine by Pratt's in 1914 which digested 58 percent of the casein in milk in 30 minutes.

N-Carbobenzoxiglycil P-nitro Phenyl Ester

Papain activity was determined with N-carbobenzoxiglycil p-nitrophenylester (Z-Gly-pNP) by the method of Kirsch & Igelström, 1966. The reaction was monitored at 400 nm and 25◦C in 0.1 M sodium phosphate buffer and 1 mM EDTA, pH 7.0, plus 6.7 % acetonitrile and ionic strength was adjusted to 0.3 M with KCl. Corrections for spontaneous hydrolysis were made. Specific activity is defined as μmol of p-nitrophenol produced per min and per mg protein under the above conditions. The molar extinction coefficient of p-nitrophenol was calculated as 9,368 M-1cm-1. Papain activity was also determined using a-N-benzoyl-L-argininethyl ester (BAEE) as substrate[44,45]. The enzyme activity ranges from 50,000 u/g to 3,500,000 u/g. Good water-solubility, without any foreign matter. For BPC grade papain, dried latex is mixed with lactose [36].

Table 3: Activity and Storage Stability of Papain Preparation

Preparation	Storage Time, Days	Activity	Related Activity %
		Abs/min	
Papain	0	0.022+/-0.004	100
	3	0.0022+/-0.001	9.9
	7	0.000/0.000	0.0
		Abs(min x g of film)	
Papain/Pectin film	0	6.409+/-0.628	100
	30	6.345+/-0.32	99
	180	6.28+/-1.696	98
Papain/Pectin/0.75% Glycerine		Abs(min x g of film)	
	0	3.974+/-0.168	100
	30	3.925+/-0.175	98
	180	3.568+/-1.103	89
		Abs(min x g of film)	
Papain/Pectin 0.25%	0	6.585+/-0.657	100
	30	1.904+/-0.13	29
	180	1.097+/-0.411	16

MODIFICATION OF PAPAIN ENZYME

The primary aim of modifying the papain is to increase the enzyme's ability to withstand alkaline pH conditions at different temperature. Baldev in 1979, reports a simple modification to the method of Kimmel & Smith (1954) that optimizes the yield of papain from highly soluble papaya latex while avoiding contamination by other enzymes[38].

Simple Modification

A solution of the latex containing 65mg of protein/ ml) is prepared by dissolving in 20mM-cysteine, pH5.7 and containing 1 mM-EDTA [47]. When spray-dried latex is used, this is readily achieved by stirring 25g of the fine white powder into 250 ml of the cysteine solution at room temperature. When the latex dissolves the pH falls to 5.3. Spray-dried latex dissolves rapidly. Then the pH of the latex solution (250ml, containing 65mg of protein/ml) is adjusted from 5.3 to 9.0 by adding 1 M-NaOH (approx. 20ml) with stirring during 10-15min at room temperature. The small amount of grey-white precipitate formed is removed by centrifugation (20000g, 4°C, and 30min) and discarded. The supernatant is adjusted to 0.45 saturation by addition of solid (NH4)2S04 (0.277 g/ml) with stirring during 20min. The white precipitate is isolated by centrifugation and dissolved in 250ml of 1 mM-EDTA. This solution is adjusted to 0.40 saturation, and again the white precipitate is isolated by centrifugation and then dissolved in 250ml of 0.1 M-phosphate buffer (KH2PO4/NaOH), pH 7.5, containing 20mM-cysteine and 1 mM-EDTA at room temperature. Solid NaCl (25g) is then added with stirring during 15min, and the precipitate is isolated by centrifugation. The white precipitate is suspended in 100 ml of pH6.5. The resulting crystals are isolated by centrifugation as above and are stored at 4°C as a suspension in 0.1 M-sodium acetate buffer, pH5.0, containing 24.3 % (w/v) (NH4)2S04. The product (usually approx. 1.5 g) when freshly prepared contains approx. 50 % active papain, 30 % reversibly blocked papain (papain-cysteine mixed disulphide) and 20 % inactive protein [38,48].

Chemical Modification and Immobilization of Papain

Papain has been chemically modified using succinic anhydride[49]. Papain does not have lysine residues in its active site, and hence it can be chemically modified easily using succinic anhydride. Succinic anhydride reacts specifically with the α-amino group of lysine residues and changes its charges from positive to negative, leading to a shift in pH optima from 6 to 8.6 Protein with negatively charged groups causes a localized lowering of pH in the surrounding liquid, thus it acquires resistance towards deactivation in highly alkaline detergent liquid. The modified papain had a specific activity of about 62.8 IUmg−1 of protein at pH 8.0 at 30 ◦C, whereas for the native enzyme it was 46.57 IUmg−1 under same conditions. Stability of the modified papain was further increased by entrapping in alginate/starch beads[50,51].

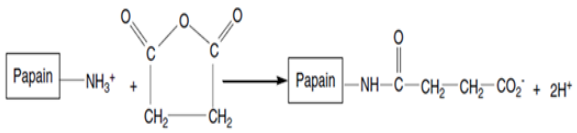

Figure 2: Succinic Anhydride Modification of Papain

Succinylation of Papain

Succinic anhydride at different concentrations from 0.4 µmol to 1 µmol was added slowly with constant stirring to papain suspension in 50 mmol dm−3 phosphate buffer, pH 8.0. This immediately brought about a drop in pH and hence 1moldm−3 NaOH was added to the solution to maintain the pH at 8.0 during the reaction. The reaction was allowed to proceed for 1h at 25◦C, after that it was stopped by lowering the pH to 7.5 using 1moldm−3 HCl. The specific activity of the succinylated papain was assayed at pH 8.0 at 40 ◦C [51].

Immobilization of Papain

Immobilization of papain by entrapment in alginate beads after chemical modification will increase its stability and reusability in alkaline conditions. For Immobilization of papain, activated papain (0.5cm3) was mixed with 4.5cm3 of 3% sodium alginate solution. The beads were formed by dropping this polymer solution into 0.2moldm−3 CaCl2 solution at room temperature. The beads were left in 0.05 mol dm−3 calcium chloride solution for 6 h to cure. The calcium alginate beads were about 2–3mm in diameter. Both native and modified papain was immobilized in alginate/ starch beads and stored in the 0.05 mol dm−3 CaCl2 solution at 40 ◦C. Starch beads were prepared using 6%(w/v) gelatinized corn starch [51].

MEASUREMENT OF MODIFIED PAPAIN ACTIVITY

The efficiency of modified papain over a pH range of 5 to 10 at 20°C after incubation of 30 min native papain loses its activity gradually above pH 6.0 and in alkaline conditions, and shows only 50% of its original activity at pH10.0 The modified papain is stable till pH 8.0, where the activity is maximum, thereafter there is only a marginal decrease in activity till pH 10.0. The activity profile of modified papain at 60°C is almost the same as at 40°C. Modified papain shows excellent stability in alkaline conditions whereas activity of the native papain gradually decreases at pH values above neutral. The activity profile of modified papain at 90°C is different from that at lower temperatures. The modified papain is stable up to pH 8.0, thereafter the activity decreases to 40 IU. Here also the modified papain is better than native papain, which loses its activity rapidly at pH values above neutral both the modified and native papain show almost the same activity till neutral pH. The native papain loses 50% of its activity at the alkaline pH of 10.0. It was found that the activity retention was 67% for modified papain and 79% for native papain and hence the enzyme did not lose much activity in 6 days. Comparison of the activity of the immobilized native and modified papain dry beads stored at room temperature is given bellow:

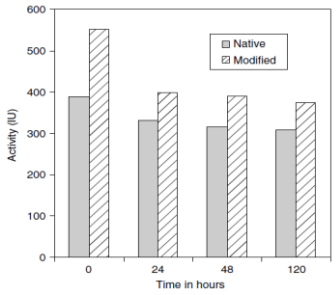

Figure 3: Comparison of the Activity of the Immobilized Native and Modified Papain Dry Beads Stored at Room Temperature

PREPARATION OF ACTIVE PAPAIN FROM DRIED PAPAYA LATEX

This method consists of preparation of a crude aqueous extract, removal of material insoluble at pH 9 which may contain papain inhibitors, $(NH4)_2SO4$ fractionation which removes another thiol-protease chymopapain, NaCl precipitation, and crystallization and re- crystallization from NaCl solution. The $(NH4)_2SO4$-fractionation step was modified in that the second precipitation was carried out at 35% instead of 40% saturation to minimize the chymopapain content of the preparation before the covalent chromatography step. A solution of the $(NH4)_2SO4$-precipitated protein was applied to the Sepharose-(glutathione-2-pyridyl disulphide) column and subjected to covalent chromatography. Separation from low-molecular-weight material by chromatography on Sephadex G-25 in the presence of 5mM-dithiothreitol yielded active papain[24]. When a mixture of fully active papain and L-cysteine was subjected to

covalent chromatography at pH4 under anaerobic conditions to prevent formation of the papain-L-cysteine mixed disulphide, all of the papain reacted with the mixed-disulphide gel and the L-cysteine passed through the column without reacting with it to an appreciable extent. This result shows the view that the reaction of papain with the gel at pH values about 4 results mainly from reaction of the unionized thiol group of papain in the cysteine-25-histidine-159-asparagine-175 hydrogen- bonded system with the mixed-disulphide gel protonated on the nitrogen atom of the pyridine ring. As the concentration of L-cysteine in the mixture to be chromatograph is increased, and the increasing proportion of L-cysteine reacts with the gel. The successful separation of papain from L-cysteine by covalent chromatography at pH4 suggests that the high reactivity that the thiol group of catalytically active papain exhibits towards 2-Py-S-S-2-Py in acidic media is exhibited also towards the mixed-disulphide gel and that this high reactivity is not exhibited by simple thiol groups[1].

THE PRODUCTION OF REFINED PAPAIN

According to Cargill Technical Services Ltd 1994, the production of refined papain using low vacuum and spray drying method was developed during 1960. The end product is fully soluble fine powder with a low bacterial count and high proteolytic activity which is between two and four times that of crude papain[3]. The production of refined papain started in Zaire in 1970 by Mr. Boudart, India 1980 and Japan 1985.T first Latex collection should be carried out in the early morning and only the oldest fruit should be tapped when they have reached 70% maturity. The area to be tapped should be divided into three sections to recover its productivity. Tapping is usually sufficient to make only one cut. The latex flows about 60-70 seconds after which it coagulates on the fruit. The coagulated materials are scrapped off by hand and collect in the tray[52]. The latex, when delivered in the 55 liter stainless steel drums is in the form of a crumbly gel. It should be inspected, weighed and spread on stainless steel tables. Extraneous matter such as dirt and insects are manually extracted before the material passes through a coarse filtration to remove the smaller foreign matter. After inspection the gelled latex is agitated at high speed with a shear stirrer in a vessel with baffles to prevent the addition of air into the liquid through the vortex formation. The material being thyrotrophic will change in consistency and become milk like liquid. The liquid is pumped into insulated, refrigerated and scaled buffer tanks of 2,500 liters each these tanks are fitted with a slow speed anchor type stirrer to keep the additives in suspension. The temperature is lowered to between 2°-5°C using chilled water circulation to prevent deterioration of the product and filter aids are added to facilitate fast filtration. The residual cake from notched filtration and plate and frame filtration is collected, mixed with water and centrifuged in a slow speed centrifuge to recover soluble active enzyme. About 8-10% soluble solid should be concentrated to between 40-50 % solid prior to drying. Drying method is performed by above techniques and the powder is collected in a cyclone separator and the fines in a wet scrubber[53].

CONCLUSIONS

Papain is a proteolytic enzymes produced from the latex of the green papaya fruits. It has high protein hydrolyzing capacity. Now a day, enzyme papain is being used in several industries.The food industries are the biggest users of papain, primarily for chill proofing of beer, tenderizing of meat and freeing of food proteins. Other applications are in tanning of leather and hides, degumming of silk, cheese manufacture, treatment of vegetable proteins, as fish hydrolysates, in treatment of fish protein concentrate and fish meals, in pharmaceuticals, aroma and perfume industries and in effluent treatment.

Papain enzymes are also understood to break down fibrin protein, which is involved in both the formation of dangerous blood clots and provides a protective coating for cancer cells. It is this action of potentially dissolving cancerous

cells protective coating that has many experts interested in papain for use in cancer prevention and possibly even as part of a treatment for cancer. Papaya enzymes are generally recognized as safe and well tolerated when used internally. There are however some potential papain side effects and specific cautions to be aware of which will be covered next.

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